Practitioner's Docket No. 81831

CHAPTER II

Preliminary Classification:
Proposed Class:
Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P., § 601, 7th ed.

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/DE00/02755	10 August 2000	12 August 1999
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

NUCLEOSIDDERIVATE UND VERFAHREN ZU DEREN HERSTELLUNG
TITLE OF INVENTION

KURT BERLIN
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

CERTIFICATION UNDER 37 C.F.R. § 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date 7 February 2002 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL919995745US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

EDWARD M. KRIEGSMAN
(type or print name of person mailing paper)

Edward M. Kriegsman
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

NOTE: To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

***WARNING:** Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

10/049177
JC10 Rec'd PCT/PTO 07 FEB 2002

2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
☐*	TOTAL CLAIMS	8 −20=	0	× $18.00=	$ 0
	INDEPENDENT CLAIMS	1 −3=	0	84.00 × ~~$78.00~~=	0
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			280 + ~~$260.00~~	280
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an international preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... $96.00 ☐ and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... $670.00 ☒ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 C.F.R. § 1.492(a)(2)) ..... $690.00 ☐ has not been paid (37 C.F.R. § 1.492(a)(3)) ..... $970.00 ☒ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) ..... 890 / ~~$840.00~~				890
				Total of above Calculations	= 1170
Applicant is a small en entity	SMALL ENTITY Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 C.F.R. § 1.9, 1.27, 1.28)				− 585
				Subtotal	585
				Total National Fee	$ 585
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL				Total Fees enclosed	$ 585

*See attached Preliminary Amendment Reducing the Number of Claims.

i. ☒ A check in the amount of $585 to cover the above fees is enclosed.

ii. ☐ Please charge Account No. _____ in the amount of $ _____. A duplicate copy of this sheet is enclosed.

***WARNING:** *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☒ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☐ has been transmitted

i. ☐ by the International Bureau.
Date of mailing of the application (from form PCT/1B/308): _____.

ii. ☐ by applicant on _____.
Date

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on _____.
Date

d. ☒ will follow.

5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. ☐ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/1B/308): _____.

ii. ☐ by applicant on (date) _____.
Date

c. ☒ have not been transmitted as

i. ☒ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210.): 28 February 2001

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the amendments were made in the English language.

c. ☒ has not been transmitted for reasons indicated at point 5(c) above.

7. ☒ A copy of the international examination report (PCT/IPEA/409)

☒ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☐ Annex(es) to the international preliminary examination report

a. ☐ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☐ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☐ is not required as the annexes are in the English language.

10. ☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on _____.
Date

b. ☐ is submitted herewith, and such oath or declaration

i. ☐ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.

XXc. ☒ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☒ is transmitted herewith.

b. ☐ has been transmitted by the International Bureau.
Date of mailing (from form PCT/IB/308): _____.

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☐ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on _____.
Date

12. ☐ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:

a. ☐ is transmitted herewith.

Also transmitted herewith is/are:

☐ Form PTO-1449 (PTO/SB/08A and 08B).

☐ Copies of citations listed.

b. ☐ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).

c. ☐ was previously submitted by applicant on _____.
Date

13. ☐ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

_____

_____

_____

14. ☐ Additional documents:
    a. ☐ Copy of request (PCT/RO/101)
    b. ☐ International Publication No. _____
        i. ☐ Specification, claims and drawing
        ii. ☐ Front page only
    c. ☐ Preliminary amendment (37 C.F.R. § 1.121)
    d. ☐ Other

_____

_____

_____

15. ☒ The above checked items are being transmitted
    a. ☒ before 30 months from any claimed priority date.
    b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____, namely:

_____

_____

_____

_____

_____

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 11-1755.

☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☐ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☐ 37 C.F.R. § 1.17 (application processing fees)

☐ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

SIGNATURE OF PRACTITIONER

EDWARD M. KRIEGSMAN

(type or print name of practitioner)

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Customer No.: 23685

10/049,177

07 June 2002

PATENT
Attorney Docket No. 81831

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
KURT BERLIN	)	
	)	
Serial No.: 10/049,177	)	Group Art Unit: Unknown
	)	
I.A. Filed: August 10, 2000	)	Examiner: Unknown
	)	
For: NUCLEOSIDDERIVATE UND VERFAHREN ZU DEREN HERSTELLUNG	)	

Box Missing Parts
Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Prior to examination of the above-identified patent application, please enter the amendment indicated below.

IN THE CLAIMS:

Please amend claims 3 and 4 as follows:

3. (Amended) The nucleoside derivatives according to claim 1, further characterized in that R_3 is an H atom, a methyl or an ethyl group.

4. (Amended) The nucleoside derivatives according to claim 1, further characterized in that R_4 is an H atom, a nitro group or a methyl group.

REMARKS

Claims 3 and 4 have been amended herein. No claims have been canceled or added herein. Therefore, claims 1-8 are under active consideration.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: _____

Edward M. Kriegsman
Reg. No. 33,529
665 Franklin Street
Framingham, MA 01702
(508) 879-3500

Dated: May 30, 2002

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box Missing Parts, Commissioner for Patents, Washington, D.C. 20231 on May 30, 2002

_____

Edward M. Kriegsman
Reg. No. 33,529
Dated: May 30, 2002

MARKED-UP AMENDED CLAIMS 3 AND 4

3. (Amended) The nucleoside derivatives according to claim 1 [or 2], further characterized in that R_3 is an H atom, a methyl or an ethyl group.

4. (Amended) The nucleoside derivatives according to [one of the preceding claims] claim 1, further characterized in that R_4 is an H atom, a nitro group or a methyl group.

Nucleoside derivatives and method for their production

The present invention concerns novel nucleoside derivatives as well as a method for their production.

Photolabile protective groups have already been described many times, particularly for the synthesis of oligomers. Particularly popular is their application to the synthesis of peptides and to the field of combinatory organic synthesis. The photolysis of protective groups is a relatively mild alternative to the traditional basic or acidic de-protection [methods] and is thus also particularly suitable for the synthesis of sensitive biomolecules. In this connection, in particular, numerous derivatives with ortho-nitrobenzyl functions have been successfully used, even for the the synthesis of oligonucleotides, e.g., on surfaces for production of oligonucleotide arrays (so-called biochips). Photo-cleavable protective groups should also be stable against basic and acidic reagents, which are applied in multi-step synthesis, and above all, they must not form highly reactive byproducts.

Nucleoside derivatives, which have been protected in the 5'-position with a derivativatized o-nitrobenzyloxycarbonyl or a 2-(o-nitrophenyl)ethoxycarbonyl function have been used almost exclusively up until now for the synthesis of oligonucleotides when photolabile protective groups are used. These functions can be effectively cleaved, for example, by irradiating with a Hg lamp, whereby

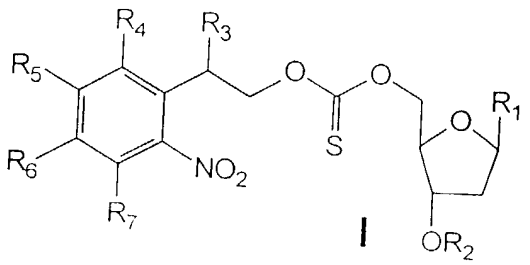
the emission line at 313 nm is a deciding factor. For example, o-nitrobenzyloxycarbonyl-protected nucleoside derivatives are also known for the commercial synthesis of oligomer arrays. Nucleoside building blocks with photolabile protective groups of the 2-(o-nitrophenyl)ethoxycarbonyl type are also known. The described protective groups, however, require relatively long irradiation times, for the most part of several minutes, for a complete cleavage of the nucleoside building block, so that secondary reactions must also be taken into consideration with sensitive biomolecules such as DNA.

Giegrich, H. et al. (Nucleosides and Nucleotides 17 (1998), pp. 1987-1996) describe the mentioned photolabile protective groups of the 2-(o-nitrophenyl)ethoxycarbonyl type. However, no indication is given that a thiocarbonyl function can also be used instead of the carbonyl function.

Photoreactive protective groups, which are of the 2(o-nitrophenyl)methoxy type are described in WO-A-94/10128. These compounds may also contain thiocarbonyl functions. The general formula, however, does not include compounds of the 2-(o-nitrophenyl)ethoxycarbonyl type.

The object of the present invention is thus to make available nucleoside derivatives, which can be easily photolysed.

The object is solved according to the invention in that nucleoside derivatives of the general formula I are created,

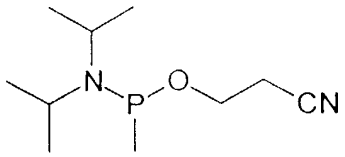


I

wherein

R_1 represents a nucleobase or a nucleobase provided with at least one protective group,

R_2 indicates an H atom or a diisopropylamino-(2-cyanoethoxy)phosphinyl group of formula IV



IV

R_3 is an H atom or an alkyl residue with up to 4 C atoms,

R_4 represents an H atom, a nitro group or an alkyl residue with up to 4 C atoms.

R_5 and R_6, independently of one another, represent an H atom, an alkyl residue with up to 4 C atoms, or an alkoxy residue with up to 4 C atoms or together represent a methylenedioxy group and

R_7 is an H atom or an alkyl residue with up to 4 C atoms.

According to the invention, it is preferred that R_1 is adenine, cytosine, guanine, thymine, uracil or hypoxanthine, which optionally bear a protective group.

In addition, it is preferred according to the invention that R_3 is an H atom, a methyl or an ethyl group.

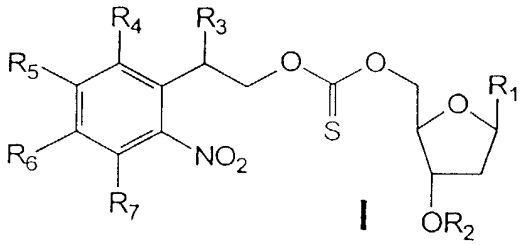
It is further preferred that

R_4 is an H atom, a nitro group or a methyl group.

It is additionally preferred that

R_5 and R_6, independently of one another, represent an H atom, a methyl, ethyl, methoxy or ethoxy group or together form a methylenedioxy group.

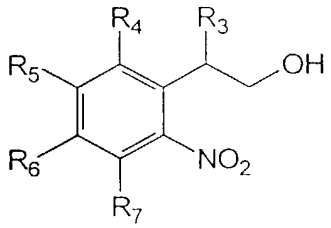
Another subject of the present invention is a method for the production of a nucleoside derivative of the general formula I



I

wherein the residues R_1, R_2, R_3, R_4, R_5, R_6 and R_7 have the above-indicated meaning,

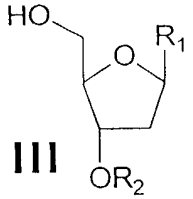
wherein a compound of the general formula II



II

wherein the residues R_3, R_4, R_5, R_6 and R_7 have the above-indicated meaning,

is reacted with thiophosgene in a way known in and of itself and the thus-obtained thiocarbonyl chlorides are reacted with a compound out the general formula III



III

wherein the residues R_1, and R_2 have the above-indicated meaning.

The present invention describes a novel type of photolabile protective groups on nucleoside derivatives (general formula I), which can be cleaved very efficiently.

The thiocarbonic acid esters corresponding to formula I can be produced in two steps, analogously to carbonic acid esters. First, a nitrobenzyl alcohol or a 2-phenylethanol derivative is reacted with thiophosgene to [form] the corresponding thiocarbonyl chloride and then coupled with the respective nucleoside building block. The nucleobase and the protective groups of the nucleoside building block have little influence on the synthesis. After introducing the photolabile protective group, the nucleoside building block can be converted into its phosphoramidite, so that it is accessible to established amidite chemistry, such as is conducted on commercial DNA synthesizers. The cleavage of the protective groups of the nucleoside derivatives according to formula I is carried out by means of irradiation with an Hg high-pressure lamp.

Another subject of the present invention is the use of the nucleoside derivatives according to the invention for the automatic synthesis of oligonucleotides. Here, automatic synthesizers and/or pipetting robots that are known in and of themselves are used in order to build up the desired oligonucleotides.

The subject of the present invention is also a kit for the automatic synthesis of oligonucleotides comprising at least one nucleoside derivative according to the invention, optionally together with other nucleoside derivatives according to the invention or already known, and reagents and adjuvants as well as solvents and operating instructions. The operating instructions may be present also in the form of a computer program for programming the automatic course of the individual synthesis steps. The desired oligonucleotides can easily be produced by means of automatic operating devices by means of this kit.

The following examples explain the invention:

Example 1:

(5'-(2-(2,6-dinitrophenyl)ethoxythiocarbonyl)thymidine

a) Preparation of 2-(2,6-dinitrophenyl)-1-ethanol

18.2 g of dinitrotoluene in 50 ml of absolute DMSO are loaded into a heated round-bottom flask and slowly mixed with a solution of 1.8 g of potassium tert-butylate in 20 ml of t-butanol. The initially slightly yellowish solution changes color to become intensely violet. The reaction mixture is first stirred at room temperature for 5 minutes and then at 70 °C for 10 minutes. It can be cooled and additionally stirred overnight at room temperature. For workup, it is neutralized with concentrated HCl and 300 ml of distilled water are added. NaCl is added to

brown oily residue remains, which is further dried in vacuum and is reacted with 2'-deoxythimidine directly with the assumption of a 100% conversion.

c) Preparation of (5'-(2-(2,6-dinitrophenyl)ethoxythiocarbonyl)thymidine

583 mg of 2'-deoxythymidine is co-evaporated three times, each time with 1.5 ml of absolute pyridine, taken up in 5 ml of absolute pyridine and cooled to -50 °C by means of an isopropanol/N_2 bath. A solution of 1 g of thiocarbonyl chloride in absolute methylene chloride is slowly dripped in, and the temperature should not increase to above -20 °C. Additional stirring is conducted overnight at room temperature. A TLC control (mobile solvent dichloromethane/methanol = 100:5) shows a clear product spot, whereupon the reaction was terminated. For the workup, the content of the flask with 50 ml of dichloromethane is transferred to a feeding funnel and washed with 50 ml of distilled water. The aqueous phase is washed 3 times with 50 ml of methylene chloride each time, and the combined organic phases are dried over $MgSO_4$. The crude product concentrated to dryness is taken up in dichloromethane/methanol (2:1), and then applied to a silica gel column; first CH_2Cl_2/MeOH = 100:5 serves as the mobile solvent, and then the MeOH gradient can be increased to 100:7 toward the end of the elution. The reaction produced the product in a yield of 363 mg (30%) as a light brown powder. Rf value (Silica 60; mobile solvent, CH_2Cl_2/MeOH 9:1) = 0.88.

Example 2:

(5'-o-(2,2-nitrophenyl)propoxythiocarbonyl)thymidine

a) Preparation of 2-(2-nitrophenyl)propanol

3.02 g (2.69 ml) of 2-nitroethylbenzene and 600 mg of paraformaldehyde in 10 ml of DMSO are loaded into a heated round-bottom flask flooded with argon and mixed dropwise with a solution of 360 mg of potassium t-butylate in 4 ml of t-butanol. After finishing the addition, stirring is conducted for 15 minutes at room temperature and then heating is conducted for 1-3/4 h at 70°C. After the solution is cooled, it is transferred with EtOAc into a feeding funnel and washed with a saturated NaCl solution. The aqueous phase is post-washed twice with EtOAc, and the combined organic phases are dried over $MgSO_4$. The crude product is purified by column chromatography. Toluene/EtOAc (8:1) serves as the solvent, and the gradient can be increased to 6:1 toward the end of the chromatography. The product is eluted only at a very late time and is spread over a broad range of the column. The reaction supplied the pure product in a yield of 2.06 g (50%) as a reddish oil, Rf value (Silica60; mobile solvent, toluene/EtOAc 8: 1) = 0.28.

b) 2-(2-nitrophenyl)propoxythiocarbonyl chloride

754 ml of thiophosgene in 15 ml of absolute THF are loaded into a heated round-bottom flask flooded with argon and provided with a septum and mixed dropwise

the end of the elution. The reaction produced the product as a light-brown foam in a yield of 937 mg (25%). Rf value (Silica 60; mobile solvent, CH_2Cl_2/MeOH 9:1) = 0.89.

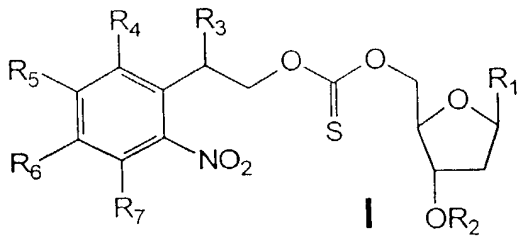
Example 3: Cleavage of the protective groups

Decomposition of 5'-o-(2-(2-nitrophenyl)ethoxythiocarbonyl)thymidine by irradiation with UV light

In order to investigate the rate of decomposition of 5'-o-(2-(2-nitrophenyl)ethoxythiocarbonyl)thymidine, 1 mg of the compound is weighed out, dissolved in 1 ml of methanol and introduced into a quartz-glass cuvette (transmission in the wavelength range of 200 nm-2500 nm, layer thickness of 1 cm). The irradiation is produced by a mercury vapor lamp of the ORIEL Instruments company, Model 66057 (output of 250 W). In order to aviod an excess heating of the cuvette, an IR filter filled with water is connected in front. The cuvette is introduced into the beam path at a distance of approximately 20 cm from the lamp optics. After a half-minute interval each time, 10 µl of the solution are removed and analyzed by means of HPLC. The measurement values are shown in Figure 1.

Patent Claims

1. Nucleoside derivatives, of the general formula I,

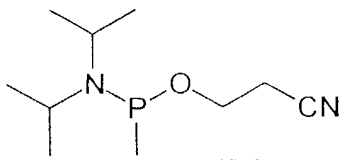


I

wherein

R_1 represents a nucleobase or a nucleobase provided with at least one protective group,

R_2 indicates an H atom or a diisopropylamino-(2-cyanoethoxy)phosphinyl group of the formula IV



IV

R_3 is an H atom or an alkyl residue with up to 4 C atoms,

R_4 represents an H atom, a nitro group or an alkyl residue with up to 4 C atoms,

R_5 and R_6, independently of one another, represent an H atom, an alkyl residue with up to 4 C atoms, or an alkoxy residue with up to 4 C atoms or together represent a methylenedioxy group,

R_7 is an H atom or an alkyl residue with up to 4 C atoms.

2. The nucleoside derivatives according to claim 1, further characterized in that R_1 is adenine, cytosine, guanine, thymine, uracil or hypoxanthine, which optionally bear a protective group.

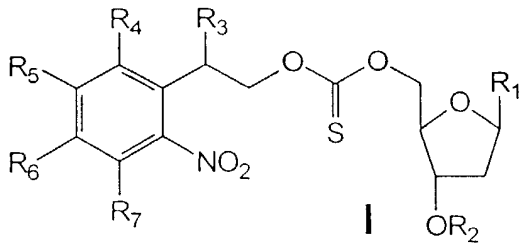
3. The nucleoside derivatives according to claim 1 or 2, further characterized in that
R_3 is an H atom, a methyl or an ethyl group.

4. The nucleoside derivatives according to one of the preceding claims, further characterized in that

R_4 is an H atom, a nitro group or a methyl group.

5. The nucleoside derivatives according to one of the preceding claims, further characterized in that
R_5 and R_6, independently of one another, represent an H atom, or a methyl, ethyl, methoxy or ethoxy group or together form a methylenedioxy group.

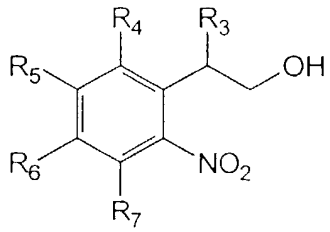
6. A method for the production of a nucleoside derivative of the general formula I



I

wherein the residues R_1, R_2, R_3, R_4, R_5, R_6 and R_7 have the meaning given in claim 1,

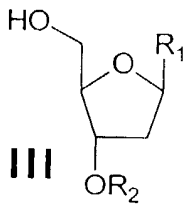
wherein a compound of the general formula II, which is known in and of itself



II

wherein the residues R_3, R_4, R_5, R_6 and R_7 as well as n [sic] have the meaning indicated in claim 1,

is reacted with thiophosgene and the thus-obtained thiocarbonyl chlorides are reacted with a compound of the general formula III



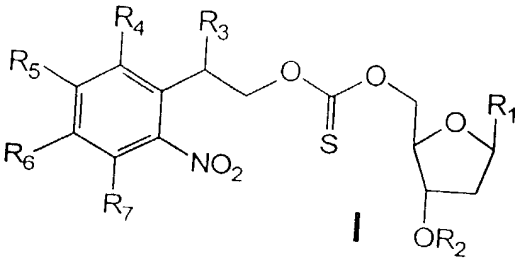
wherein the residues R_1, and R_2 have the meaning indicated in claim 1.

7. Use of the nucleoside derivatives according to claim 1 for the automatic synthesis of oligonucleotides.

8. A kit for the automatic synthesis of oligonucleotides comprising at least one nucleoside derivative according to claim 1, optionally together with other nucleoside derivatives according to claim 1 and reagents and adjuvants as well as solvents and operating instructions.

Abstract

Novel nucleoside derivatives, of general formula I are described:



I

wherein

R_1 represents a nucleobase or a nucleobase provided with at least one protective group,

R_2 indicates an H atom or a diisopropylamino-(2-cyanoethoxy)phosphinyl group,

R_3 is an H atom or an alkyl residue with up to 4 C atoms,

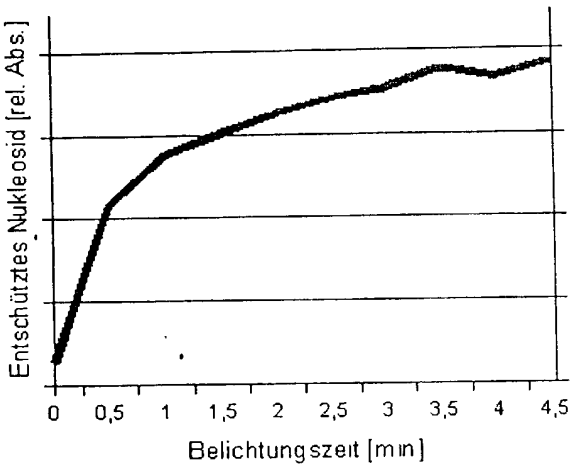
R_4 represents an H atom, a nitro group or an alkyl residue with up to 4 C atoms.

R_5 and R_6, independently of one another, represent an H atom, an alkyl residue with up to 4 C atoms, or an alkoxy residue with up to 4 C atoms or together represent a methylenedioxy group, and

R_7 is an H atom or an alkyl residue with up to 4 C atoms.

The new nucleoside derivatives can be easily cleaved by means of UV light and are used for the synthesis of oligonucleotides.

Fig. 1



y-axis: deprotected nucleoside [rel. units]

x-axis: exposure time [min]

Attorney Docket No. 81831

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled, NUCLEOSIDDERIVATE UND VERFAHREN ZU DEREN HERSTELLUNG, the specification of which: (check one)

[ ] is attached hereto.
[X] was filed as PCT Application No. PCT/DE00/02755 on August 10, 2000 and has been assigned U.S. Patent Application Serial No: 10/049,177.
[ ] was filed on ;
and assigned Serial No.:

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed
19938092.9	Germany	12 August 1999	[X] yes [ ] no
(number)	(country)	(day/month/year filed)	
			[ ] yes [ ] no
(number)	(country)	(day/month/year filed)	

I hereby claim the benefit under Title 35, United States Code, 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE:

81831

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations 1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(application number)	(filing date)	(Status - patented, pending, abandoned)
(application number)	(filing date)	(Status - patented, pending, abandoned)
(application number)	(filing date)	(Status - patented, pending, abandoned)
(application number)	(filing date)	(Status - patented, pending, abandoned)

I hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Irving M. Kriegsman, Esq., Reg. No. 22,733; Edward M. Kriegsman, Esq., Reg. No. 33,529; and Daniel S. Kriegsman, Esq., Reg. No. 40,057.

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I hereby declare that all statements made herein of my own knowledge are true and that any statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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